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Proc. R. Soc. Lond. B 1906 **78**, 369-375
doi: 10.1098/rspb.1906.0070

References

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The Alcoholic Ferment of Yeast-juice. Part II.—*The Coferment of Yeast-juice.*

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(Communicated by Dr. C. J. Martin, F.R.S. Received June 14,—Read June 28, 1906.)

(From the Chemical Laboratory, Lister Institute.)

In a previous communication* it was shown that the fermentation of glucose by yeast-juice is dependent upon the presence of a dialysable substance which is not destroyed by heat. This substance is contained in the active yeast-juice prepared by disintegrating living yeast, and, therefore, most probably exists in the yeast cell side by side with the zymase.

The occurrence of an analogous activating substance has been described by Magnus† in the case of the lipase of the liver. He observed that the active juice of this organ became inactive when dialysed into water, but regained its activity when the dialysate or boiled liver juice was added. The term *coferment* was suggested by Bertrand‡ to denote substances of this kind, and he applied it in two instances—to the calcium salt, which he considered was necessary for the action of pectase on pecten substances, and to the manganese of laccase, which he supposed to be essential for the activity of this enzyme. Although not entirely satisfactory, this term may be provisionally applied to activating substances such as those present in liver lipase and yeast-juice, until further knowledge of their nature and function permits of a more rational terminology.

(1) *Preparation of the Inactive Residue from Yeast-juice in the Dry State.*

In the previous communication§ it was shown that when yeast-juice is filtered through a Martin gelatin filter, both the residue and the filtrate are incapable of fermenting sugar, whereas a mixture of the two produces a vigorous fermentation.

In carrying out the experiments which established this result, the residue left on the filter was always dissolved in water as soon as the filtration and washing were completed, and the activity of the solution was examined without delay. It has since been found possible to obtain the inactive

* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 410.
† 'Zeit. Physiol. Chem.,' 1904, vol. 42, p. 149.
‡ 'Compt. Rend.,' 1897, vol. 124, p. 1032.
§ *Loc. cit.*

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residue in the solid form, in which condition it retains its properties for a considerable time.

This is accomplished by spreading the sticky mass left on the filter over a large watch-glass, and exposing it over sulphuric acid in a vacuum. The residue dries up in a few hours to a brittle mass, which is converted by grinding into a light yellow powder.

Complete removal of the coferment is generally not effected by a single filtration, and the powder prepared as above is usually found to be slightly active. A completely inactive residue may, however, be obtained by redissolving in water and repeating the filtration and desiccation. The powder prepared in this way and dried over sulphuric acid in a vacuum for 15 hours only loses its potential activity slowly. The following examples show the original inactivity of the powder, its fermentative power in presence of boiled yeast-juice, and the loss of this power with lapse of time :—

I. 200 c.c. of yeast-juice were filtered in quantities of 50 c.c. through Martin filters and yielded 17·6 grammes of dry solid. This was dissolved in 50 c.c. of water and again filtered, yielding 9·2 grammes of dry solid residue.

The following experiments were then made at 26° in presence of toluene :—

1. March 8, 1906. One gramme of dry residue was dissolved in 15 c.c. of water and 1·5 grammes glucose added. No evolution of carbon dioxide was observed; 12 c.c. of boiled yeast-juice containing 1·2 grammes of glucose were then added. Fermentation at once commenced, and 108 c.c. of carbon dioxide were evolved in 20 hours, when the experiment was discontinued.

2. Two months later (May 18, 1906) a similar experiment was carried out, a sample of the same boiled yeast-juice being employed. As before, 1 gramme of the residue dissolved in sugar solution was quite inactive. On the addition of the boiled juice fermentation at once commenced; the rate was, however, only about one-third of that attained in the previous experiment, and fermentation ceased when only 42 c.c. of carbon dioxide had been evolved.

Although the fermentation in the first experiment was not continued to the end, the comparison shows clearly that the potential activity of the dry residue had greatly diminished.

II. In the case of another sample of dry residue, with which two similar experiments were made with the interval of a week, it was found that in the first experiment 364 c.c. of carbon dioxide were produced, and in the second 344 c.c., a difference of only about 5·5 per cent.

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(2) *Disappearance of the Coferment from Yeast-juice during Fermentation and Autolysis.*

When a small amount of boiled yeast-juice is added to a solution of the inactive residue in 10-per-cent. glucose solution, fermentation commences and continues for a period which varies with the amount added. The cessation of fermentation appears in such a case to be due to some change in the coferment, since the addition of a further quantity of boiled yeast-juice again sets up fermentation, and if the quantity of boiled juice added on each occasion be small enough, this process can be repeated a third time.

The following experiments, made at 26° in presence of toluene, illustrate this point:—

1. One gramme of dry inactive residue was dissolved in 15 c.c. of 10-per-cent. glucose solution, and three quantities of 3 c.c. of boiled juice were then successively added, the fermentation being allowed to come to an end before each addition.

	Carbon dioxide evolved.
1st addition	8·2 c.c.
2nd „	6 „
3rd „	6 „

2. Two grammes of dry inactive residue were dissolved in 15 c.c. of 10-per-cent. glucose solution, and two quantities of 15 c.c. of a diluted boiled juice containing 10 per cent. of glucose were successively added.

	Carbon dioxide evolved.
1st addition.....	54 c.c.
2nd „	41·2 „

This phenomenon has been frequently observed in the course of experiments on the filtration of the juice through the Martin filter. The first residue obtained is generally slightly active, and produces a small amount of fermentation with glucose solution. The evolution of carbon dioxide, however, soon stops, but is again renewed when boiled yeast-juice is added. One instance of this was quoted in our previous communication,* and many more have been observed, some of which are tabulated below.

Carbon dioxide evolved from residue + glucose.	Boiled juice added.	Subsequent evolution of carbon dioxide.
c.c.	c.c.	c.c.
8·6	20	72
13·2	16	364·3
16·4	20	368·8

* *Loc. cit.*, p. 410.

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The cessation of fermentation in any particular mixture of inactive residue and coferment may, therefore, be due to the disappearance either of ferment or coferment from the liquid. If the amount of coferment present be relatively small, it is the first to disappear, and fermentation can then only be renewed by the addition of a further quantity, whilst the addition of more ferment produces no effect. If, on the other hand, the amount of coferment be relatively large, the inverse is true; the ferment is the first to disappear, and fermentation can only be renewed by the addition of more ferment, a further quantity of coferment producing no effect. This is illustrated by the following experiment made at 26°:—

Two solutions were made up, each containing 0·5 gramme of dry inactive residue +10 c.c. of 10-per-cent. glucose solution and toluene, and to each of these 2 c.c. of boiled juice containing 10 per cent. glucose were added. As soon as fermentation had ceased, 0·5 gramme of dry residue in 10 c.c. of glucose solution was added to one solution (*a*), and 2 c.c. of boiled juice diluted to 10 c.c. with glucose solution to the other (*b*). Two other solutions were also prepared, each containing 0·4 gramme of the same dry inactive residue +10 c.c. of 10-per-cent. glucose solution + toluene, and 25 c.c. of the same boiled juice containing glucose were added to each.

As soon as fermentation had ceased, 0·4 gramme of residue dissolved in 10 c.c. of glucose solution was added to one (*c*), and 10 c.c. of boiled juice containing glucose to the other (*d*). The following were the results:—

	Boiled juice added.	Fermentation produced.	Subsequent addition.	Additional fermentation.
	c.c.	c.c.		c.c.
(*a*)	2	9·4	0·5 gr. residue	0
(*b*)	2	7·6	2 c.c. boiled juice	8·9
(*c*)	25	56·8	0·4 gr. residue	36·8
(*d*)	25	50	10 c.c. boiled juice	0

(3) *Rate of Disappearance of the Coferment from Yeast-juice.*

The following experiments were made to ascertain approximately the rate at which the coferment disappears from yeast-juice both in the absence and presence of added glucose. For this purpose a quantity of yeast-juice was preserved at 25° in presence of toluene, and samples were removed, boiled and filtered, at the commencement of the incubation and after various intervals. In one case a parallel experiment was made with yeast-juice to which 10 grammes of glucose per 100 c.c. had been added. The filtrates were then all rendered neutral to litmus and tested with equal quantities of an inactive

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residue and glucose, care being taken to keep the concentration of glucose the same throughout.

Material digested.	Time of digestion.	Volume of filtrate taken.	Fermentation produced.
	days.	c.c.	c.c.
1. *a.* Yeast-juice alone	0	20	168·5
b. „	9	20	0
2. *a.* „	0	16	364·3
b. „	2	16	2·6
c. „	4	16	0
3. *a.* „	0	15	62·9
b. „	1	15	2·1
c. „	2	15	0
d. „	4	15	0
e. Yeast-juice + glucose.....	0	15	62·9
f. „	1	15	15·6
g. „	2	15	4·5
h. „	4	15	8·3

In two cases in which a negative result was obtained (1*b* and 2*c*) an equal volume of the same fresh boiled juice was added and in both cases a vigorous fermentation was produced, thus proving that the autolysed juice had not exerted any specific detrimental effect on the ferment:—

	Fresh boiled juice added.	Carbon dioxide evolved.
1. *b*.....	20 c.c.	112 c.c.
2. *c*.....	16 „	297 „

It appears from this that the coferment disappears from the juice less rapidly in the presence of glucose than in its absence. In yeast-juice to which no addition of glucose has been made the coferment usually disappears at 26° in about 48 hours; in the presence of 10 per cent. of glucose coferment was still present at the end of four days, although only in a small amount. (The observation that the fermentation after four days' incubation is somewhat greater than that given after two days is probably to be explained by the presence of a greater amount of phosphate in the juice which had been digested for the longer period.)

4. *Soluble Phosphates do not Render the Inactive Residue Capable of Fermenting Glucose.*

In view of the fact that soluble phosphates, as described in the previous communication, exert a remarkable effect on the fermentation of glucose by yeast-juice, experiments were made to ascertain whether the addition of a soluble phosphate to a solution of the inactive residue in glucose is sufficient

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to set up fermentation. All the attempts hitherto made to effect this have yielded entirely negative results, although both the kind of phosphate and the amount added have been varied.

Dipotassium hydrogen phosphate, a mixture of this with the dihydrogen phosphate, disodium hydrogen phosphate, diammonium hydrogen phosphate, microsmic salt, and a mixed phosphate of potassium and magnesium obtained by boiling a solution of potassium dihydrogen phosphate with magnesium carbonate, were employed, all of which are capable of producing the characteristic effect of phosphates on the fermentation of glucose by yeast-juice.

Although these substances did not set up fermentation when added to an inactive filtered residue and glucose, they did not affect the potential activity of the residue. This is evident from the fact that the subsequent addition of boiled juice produced an immediate fermentation.

In every case the solution of the phosphate was saturated with carbon dioxide at 26° and added to the solution of the inactive residue in glucose solution also saturated with carbon dioxide at 26°, and in no case was any evolution of gas observed after the cessation of the slight disturbance which inevitably occurs when the solutions are mixed. The phosphate solutions were all of 3/10 molar strength, with the exception of the mixed phosphate of magnesium and potassium, 5 c.c. of which yielded 0·1594 gramme $Mg_2P_2O_7$.

In those cases in which the residue employed was slightly active, incubation was continued until all fermentation had ceased before the phosphate solution was added, and the amount of carbon dioxide evolved in this preliminary period is given in the table. The amount of glucose, both in the solution of the residue and in that of the phosphate, was throughout 10 grammes to 100.

	Amount of residue.	Preliminary fermentation.	Phosphate added.	Carbon dioxide produced.	Subsequent evolution of carbon dioxide after the addition of boiled juice.
1	25 c.c.	41·2	2·5 c.c. KMg phosphate ...	0	18·4
2	25 „	47·7	5 „ „ ...	0	—
3	25 „	0·6	3 „ K_2HPO_4	0	268·8
4	1·6 gr.	21·5	3 „ KMg phosphate ...	0	159·2
5	1·2 „	10·7	5 „ „ ...	0	—
6	0·5 „	0	2 „ mixture of K_2HPO_4 and KH_2PO_4	0	3 c.c. per hour
7	0·5 „	0	2 „ $(NH_4)_2HPO_4$	0	4·2 „ „
8	0·5 „	0	2 „ $(NH_4)NaHPO_4$ ...	0	3·8 „ „
9	0·5 „	0	2 „ Na_2HPO_4	0	3·6 „ „

The foregoing conclusion is confirmed by the observation, made in the

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experiments on the disappearance of the coferment from yeast-juice, that boiled autolysed juice does not set up fermentation in a mixture of the inactive residue with glucose, although it itself contains a large amount of phosphate precipitable by magnesia mixture. The following numbers were obtained by the analysis of three specimens of boiled autolysed juice employed in those experiments, two of which were quite inactive, whilst the other only produced a fermentation of 2·6 c.c.

No. of experiment.	Volume of juice.	Carbon dioxide evolved.	Phosphate present in grammes of $Mg_2P_2O_7$.
	c.c.	c.c.	
1. *b*	20	0	0·3400
2. *b*	16	2·6	0·3011
3. *c*	15	0	0·1893

These experiments throw no light on the actual chemical nature of the coferment, but show that most probably it does not consist of a phosphate precipitable by yeast-juice. They also indicate that substances, which, like phosphates, increase the total fermentation produced by yeast-juice, are not necessarily capable of setting up fermentation when added to a mixture of inactive residue and glucose.

Further experiments are in progress with the hope of obtaining more information on these points.